

1 **Towards the structure of the TIR-domain signalosome**

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16 **SUMMARY**

17 TIR (Toll/interleukin-1 receptor/resistance protein) domains feature in animal, plant and bacterial
18 proteins involved in innate immunity pathways and associated processes. They function through
19 protein:protein interactions, in particular self-association and homotypic association with other TIR
20 domains. Structures of TIR domains from all phyla have been determined, but common association
21 modes have only emerged for plant and bacterial TIR domains, and not for mammalian TIR
22 domains. Numerous attempts involving hybrid approaches, which have combined structural,
23 computational, mutagenesis and biophysical data, have failed to converge onto common models of
24 how these domains associate and function. We propose that the available data can be reconciled in
25 the context of higher-order assembly formation, and that TIR domains function through signaling
26 by cooperative assembly formation (SCAF).

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29 INTRODUCTION

30 The TIR (Toll/interleukin-1 receptor (IL-1R)/resistance protein) domain was first defined after
31 detecting sequence similarities between the intracellular regions of the mammalian IL-1R and
32 the *Drosophila* protein Toll [1]. TIR domains typically function as protein interaction modules, and
33 are mostly found in multi-domain proteins involved in innate immunity pathways in animals and
34 plants, despite the proposed independent evolutionary origins for these pathways [2]. TIR domains
35 also appear in many bacterial proteins, at least some of which are used by pathogenic bacteria to
36 evade the host immune responses [3].

37 In mammals, TIR domains are found in Toll-like receptors (TLRs) and IL-1Rs as their
38 cytosolic segments, as well as in the cytosolic adaptor proteins involved in signaling downstream
39 from these receptors. TLRs (10 family members in humans: TLR1-10) are pattern-recognition
40 receptors (PRRs) that defend against microbial infection and endogenous danger, by interacting
41 with conserved pathogen- and danger-associated molecular patterns (PAMPs/DAMPs) [4]. These
42 interactions lead to the TLR-selective recruitment of the TIR domain-containing adaptor proteins
43 MyD88, MAL (TIRAP), TRIF (TICAM-1) and TRAM (TICAM-2) via TIR:TIR domain
44 interactions [5]; these interactions trigger downstream activation of transcription factors such as
45 NF- κ B, AP-1 and IRFs to induce anti-pathogen signaling and inflammation [6]. An atypical TLR
46 adaptor is SARM, which acts as a negative regulator of TRIF signaling [7], but also functions in
47 neuronal axon degeneration [8,9] and cell-death pathways [10]. BCAP (B-cell adaptor for PI3K)
48 has recently been proposed to be the sixth TIR domain-containing TLR adaptor [11,12]. IL-1Rs (10
49 family members found in humans: IL-1RI, IL-1RII, IL-1RaCP, ST-2, IL-1Rrp, IL-1Rrp2, IL-
50 1RAcPL, IL-1RAPL, IL-1RAPL2 and SIGIRR) associate with proinflammatory cytokines, and like
51 some of their TLR cousins, signal by recruiting the TIR domain-containing adaptor MyD88 [13].

52 In plants, TIR domains are found as the N terminal segments of a major subclass of
53 cytoplasmic nucleotide-binding (NB)/leucine-rich repeat (LRR) resistance (R) proteins. NB-LRR
54 proteins are typically referred to as plant NLRs due to their similarity to mammalian nucleotide-

55 binding oligomerization domain (NOD)-like receptors [14]. Plant NLRs directly or indirectly
56 recognize "effector" proteins introduced into the plant cell by plant pathogens during the invasion
57 of the plant. Effector detection by plant NLRs triggers defense responses, known as the
58 hypersensitive response, that often include localized cell death at the site of infection [15]. The TIR
59 domains are considered to be the signaling domains in plant NLRs, because they can cause cell
60 death autonomously when expressed ectopically *in planta* [16-18]. TIR-only (TIR-X) and TIR-NB
61 (TIR-N) proteins are also found in plants [19], and while their general functions are to date
62 unknown, a number of these proteins have been shown to induce cell death when transiently
63 expressed in tobacco and provide enhanced resistance when overexpressed in stable transgenics in
64 *Arabidopsis* [20].

65 TIR domains are also found in proteins from a wide range of bacterial species, where they
66 exist in combination with different types of domains [3]. Although the functions of most of these
67 proteins are unknown, some proteins such as TcpB from *Brucella melitensis* and TcpC from
68 uropathogenic *Escherichia coli* CFT073 suppress TLR signaling, possibly through interacting with
69 the host TIR domain-containing proteins [21].

70 In all these different organisms, TIR domains are thought to function through self-
71 association and homotypic association with other TIR domains. However, they can also engage in
72 heterotypic interactions with proteins not containing TIR domains (e.g. the vaccinia virus protein
73 A46 can bind MyD88, MAL, TRIF, TRAM and TLR4 [22]), and in intramolecular fashion with
74 other domains in TIR domain-containing proteins [14] (e.g. with both the NB and LRR domains in
75 the plant NLR RPP1 [23], and with an N-terminal helix in the bacterial protein TcpB [24]).

76 Currently, 32 structures corresponding to 16 different TIR domains from animals, plants and
77 bacteria have been deposited in the Protein Data Bank [25]. Structurally, TIR domains comprise
78 125-200 residues and contain a central parallel β -sheet surrounded by α -helices [25,26]. The
79 elements of secondary structure are usually referred to sequentially; for example the BB loop
80 connects strand β B with helix α B. Some of these structural elements correspond to conserved

81 sequence motifs called box 1–3 in mammalian TIR domains [25]. While the wealth of structural
82 information has improved our understanding of TIR-domain function in individual systems, it is
83 widely assumed that TIR-domain functions in different systems do not converge on a common
84 mechanism of action. To date, no common self-association interfaces have been observed in the
85 crystal structures of animal TIR domains, and numerous studies combining structural knowledge of
86 TIR domains with computational docking, site-directed mutagenesis and other methods have
87 proposed models that are different from each other [27-48]. By contrast, some common association
88 modes are emerging for plant and bacterial TIR domains. Here, we review the key studies
89 attempting to define the structural basis of TIR-domain function and suggest that both in plant and
90 mammalian innate immunity pathways, it could be explained in the context of signaling by
91 cooperative assembly formation (SCAF) (Box 1).

92

93 **2. SELF-ASSOCIATION AND HOMOTYPIC ASSOCIATION OF TIR DOMAINS IN MAMMALIAN PROTEINS**

94 TLR and IL-1R-dependent signal transduction is initiated by self-association of their intracellular
95 TIR domains (hereafter denoted with superscript "TIR") upon binding of PAMPs (TLRs) or
96 cytokines (IL-1Rs). The TLR^{TIR} dimer then acts as a scaffold to recruit downstream adaptor
97 proteins through TIR:TIR domain interactions. The highly conserved BB-loop in TLR/IL-1R and
98 adaptor TIR domains plays an important role in signaling. In TLR4, the BB loop is the site of a
99 naturally occurring mutation P712H [49], which renders it non-responsive to the PAMP
100 lipopolysaccharide (LPS). This mutation also abolishes signaling when introduced into other
101 receptor or adaptor TIR domains.

102 MyD88 also contains a death domain (DD) that interacts with IRAKs (IL-1R-associated
103 kinases) through DD:DD interactions, forming the oligomeric myddosome, consisting of six
104 MyD88, four IRAK4 and four IRAK2 DDs [50] (Box 1c). Forced dimerization of MyD88^{TIR}
105 constitutively initiates signaling [51], suggesting that upon TLR activation, the TLR, MAL and
106 MyD88 form an oligomeric platform through TIR:TIR domain interactions, which in turn promotes

107 the assembly of the myddosome via DD:DD interactions. In comparison to MyD88 signaling, less
108 is known about TRIF signaling, but live-cell imaging and confocal immunofluorescence analyses
109 have shown that TRIF alters its distribution profile from a diffuse cytoplasmic to a speckle-like
110 structure in response to TLR3 interaction with dsRNA [52], suggesting the formation of TIR
111 domain-dependent oligomeric TRIF complexes.

112 Crystal structures have been determined for the TIR domains of human TLR1, TLR2,
113 TLR6, TLR10, IL-1RAPL, MAL, MyD88 [26,30,37,40-43,53] and Toll-related receptor TRR-2
114 from the lower metazoan *Hydra magnipapillata* (PDB ID 4W8G, 4W8H). NMR structures have
115 also been determined for MyD88^{TIR}, TRAM^{TIR} and TRIF^{TIR} [32,36]. Attempts to form stable TIR-
116 domain complexes have been unsuccessful, suggesting that weak interactions are a general feature
117 of the mammalian TIR-domain complexes, and that membrane localization or the context of a large
118 assembly stabilizes the interactions. Crystal contacts can reflect biological interactions [54];
119 analyses of crystal structures and combinations of computational modeling and docking studies,
120 NMR and site-directed mutagenesis have led to several models of TIR domain assembly and
121 although they are all different from each other [27-48], some common trends in the proposed
122 TIR:TIR domain interaction modes are emerging (Figure 1, Table S1).

123 *The BCD interface.* Several of the crystal structures (TLR1, TLR2, TLR6, IL-RAPL, MAL
124 and TRR-2) contain an interface involving the α C helices and either the α B/BB-loops or the α D
125 regions, or both (the BCD interface) (Figure 1). In the TLR1^{TIR}, TLR2^{TIR} and TLR6^{TIR} structures,
126 symmetric α C: α C helix interactions are found at the core of this interface, flanked on both sides by
127 interactions between the BB-loop/ α B region on one molecule and the DD-loop/ α D region on the
128 second molecule [26,43]. It has been questioned whether this interface is physiologically relevant,
129 because in both TLR1 and TLR6, it is stabilized by a disulfide bond (between the C707 residues in
130 TLR1 and the equivalent C712 residues in TLR6). However, a similar interface involving the same
131 secondary structure elements is also observed in the IL-1RAPL crystal structure [30]. In the
132 TLR10^{TIR} dimer [40], one of the molecules has been rotated 90° compared to the TLR1^{TIR}, TLR2^{TIR}

133 and TLR6^{TIR} dimers, resulting in the two BB loops of TLR10^{TIR} interacting directly with each
134 other. Many loss-of-function mutations in TLR4 localize to the surface regions involved in this
135 interface and the TLR10^{TIR} homodimer has therefore been widely accepted as representative of
136 TLR4^{TIR} dimerization following LPS recognition [27-29,31,36].

137 Crystal-contact analysis of the MAL structures revealed a symmetric interface comprising
138 the α C and α D regions. Mutations of residues in this interface disrupt both MAL and MyD88
139 binding [37,41]. In one of the crystal forms of TRR-2^{TIR} (PDB ID 4W8G), one of the molecules has
140 been rotated 180° compared to the MAL dimer, and the interface consists of the α C and α D helices
141 of one molecule and the α C and α B helices of the second molecule. Although significant
142 differences are observed between the interfaces described here, they are all centered around the α C
143 helix and involve similar faces of the TIR domain. Furthermore, docking of TRAM^{TIR} NMR
144 structures, using data based on mutagenesis coupled with yeast-two-hybrid (Y2H) assays as
145 restraints, suggested that TRAM^{TIR} can self-associate using a similar configuration to the TLR10^{TIR}
146 dimer [36], while MyD88^{TIR} can self-associate via a MAL^{TIR}-like dimer interface [35].

147 *The BE interface.* The MyD88^{TIR} crystal structure and the two different crystals forms of
148 TRR-2^{TIR} contain an asymmetric head-to-tail TIR:TIR domain interaction involving the BB-loop of
149 one molecule and the surface encompassing the β E/EE loop/ α E region of the second molecule (the
150 BE interface; Figure 1c). Extensive mutagenesis using the mammalian-two-hybrid (MAPPIT)
151 methodology combined with docking also provides support for an asymmetric BE interface
152 involved in MyD88 self-association [35]. Furthermore, site-directed mutagenesis data identify both
153 the BB-loop (R196/D197) and helix α E (K282/R288) as MAL-binding sites, suggesting that
154 MAL^{TIR} and MyD88^{TIR} may interact through a similar head-to-tail mode [32].

155 Some lines of evidence suggest that purified TLR adaptor TIR domains may form higher-
156 order oligomers at high protein concentrations. For example, the ¹⁵N-labeled signals from
157 MyD88^{TIR} uniformly decreased upon titration with MAL^{TIR} [32]. Furthermore, TRAM and TRIF

158 oligomerized and precipitated out of solution at concentration above 200 μ M [36]. Precipitation was
159 prevented by the introduction of a BB-loop mutation (C117H in TRAM and P434H in TRIF),
160 which has previously been shown to disrupt self-association in Y2H assays and to have a dominant
161 negative effect in IFN- β reporter assays; this enabled the NMR structures of the monomeric
162 proteins to be determined.

163 Many of the TIR-domain assembly models have assumed a 2:1 or 1:1 receptor/adaptor
164 TIR:TIR domain stoichiometry [27,28,31], but more recent models try to rationalize how a single
165 TLR^{TIR} dimer can recruit >6 MyD88 molecules required for myddosome assembly. In one study
166 [29], the PRISM algorithm combined with existing crystal structures and experimental data was
167 used to model MyD88 and TRIF signalosomes. Several different plausible models are presented,
168 but it is argued that the most likely is a model consisting of a symmetric BCD-interface TLR4
169 dimer (similar to the TLR10^{TIR} dimer) that interacts with two symmetric BCD-interface MAL
170 dimers, which in turn recruit two symmetric MyD88 dimers; this would result in clustering of 8
171 MyD88 DDs, enabling myddosome formation. A completely different model, based on MAPPIT
172 mutagenesis data and docking, is presented in another study [35], where it is proposed that MyD88
173 oligomerization is a result of self-association through both a symmetric BCD interface (similar to
174 the MAL^{TIR} crystal dimer) and an asymmetric BE interface. By combining the two types of
175 interactions, it is proposed that MyD88^{TIR} molecules can assemble into a left-handed helix, bringing
176 the DDs together for myddosome assembly. This model displays similarities to the open-ended
177 pyrin domain (PYD)/CARD assemblies recently described for other innate immunity pathways (e.g.
178 the inflammasomes [55] and MAVS-dependent RIG-I/MDA-5 signaling [56]), and extension of the
179 left-handed helix would presumably enable a single TLR dimer to assemble multiple myddosomes,
180 which is consistent with the ability of TLRs to activate a large transcriptional response based on
181 extremely low concentrations of PAMPs. Although this model is consistent with observed TIR:TIR
182 domain interaction modes, the observed variations could give rise to different oligomeric TIR-
183 domain architectures. For example, in one of the TRR-2 crystal forms (PDB ID 4W8G), we also

184 observe a combination of BCD and BE interfaces, which results in a formation of a linear parallel
185 two-stranded head-to-tail array of TIR domains within the crystal (Figure 1d). This architecture
186 would also enable MyD88 DD clustering and myddosome formation. The BCD interface in this
187 linear assembly differs from the MAL-based BCD interface in [35] by a 180° rotation of one of the
188 molecules. However, it involves the α C and α D helices and can thus explain the reported MyD88
189 mutagenesis data. Our analyses illustrate that care must be used in interpreting docking results with
190 limited structural information, and to fully elucidate the molecular mechanisms of TIR-domain
191 assembly formation and the exact nature of the interfaces, structural information on stable
192 oligomeric assemblies will be required. Furthermore, TIR-domain proteins usually contain other
193 domains and can be attached to membranes; however, the TIR-domain linker sequences are usually
194 of sufficient length (>20 residues) to enable the proposed interactions on cell-membranes or in the
195 presence of other domains.

196

197 **3. SELF-ASSOCIATION AND HOMOTYPIC ASSOCIATION OF TIR DOMAINS IN PLANT PROTEINS**

198 The Arabidopsis TIR-X protein AtTIR (AT1G72930) provided the first plant TIR-domain structure
199 [57]. It revealed a similar fold to those observed for mammalian TIR domains; however, an
200 extended α D region is found. This feature appears to be unique to the plant TIR domains and
201 present in most, but not all. AtTIR was reported to be monomeric in solution [57]; however, this data
202 was inferred from size-exclusion chromatography (SEC) alone, which, as subsequent studies have
203 revealed, is unlikely to detect transient self-association. The first TIR-domain structure from a plant
204 NLR came from the flax protein L6. L6^{TIR} can self-associate according to Y2H and in-solution
205 assays (SEC/multi-angle laser light scattering (MALS) and analytical ultracentrifugation (AUC))
206 [17]. Crystal-contact analysis, combined with mutagenesis, in-solution self-association assays and
207 Y2H assays, revealed that the α D_{1/3}, β E and α E regions mediate L6^{TIR} self-association (the DE
208 interface; Figure 2a, Table S1). Self-association is linked to the cell death-inducing activity
209 association of L6^{TIR} [17].

210 The RPS4^{TIR}:RRS1^{TIR} complex is the only crystal structure available for a complex of two
211 different TIR domains. RPS4 and RRS1 are jointly responsible for NLR-mediated resistance to
212 three different pathogens in Arabidopsis. The regions that mediate the heterodimer interaction (α A,
213 α E and the AA and EE loops - the AE interface) are also observed in the structures of RRS1^{TIR},
214 RPS4^{TIR} and AtTIR as individual proteins [18]. RPS4^{TIR}, but not RRS1^{TIR} can induce cell-death
215 signaling responses. The AE interface has been recently also observed in the crystal structures of
216 the TIR domains from the wild grape NLR RPV1 [58] and the Arabidopsis NLRs SNC1 [59,60]
217 and RPP1 [60].

218 The self-association of plant TIR domains observed to date is weak and transient; the
219 dissociation constants measured for L6^{TIR} and RPS4^{TIR} by AUC experiments are in the high μ M
220 range. RPV1^{TIR} did not appear to self-associate *in vitro* under the conditions tested. It is speculated
221 that TIR:TIR domain interactions would be stabilized, in the activated NLRs, by self-association of
222 other domains such as the NB domains, based on comparisons with the related mammalian NLRs
223 [14]. By contrast, the heterodimer formed between RPS4^{TIR}:RRS1^{TIR} is \sim 100x stronger (455 nM)
224 than any self-associations of plant TIR domains. RRS1^{TIR} suppresses RPS4^{TIR} cell-death signaling
225 in plants and suggests that the RPS4^{TIR}:RRS1^{TIR} interaction represents a repressed state of the pair
226 [18].

227 The interfaces that mediate self-association in L6^{TIR} and RPS4^{TIR} are distinct, but they could
228 co-exist (Figure 2). Mutations in Arabidopsis RPP1^{TIR} that map to both the DE and AE interfaces
229 affect RPP1^{TIR} self-association, and a correlation between the degree of self-association *in vitro* and
230 cell-death signaling has been observed [23]. These data suggest that both interfaces may facilitate
231 self-association and signaling in RPP1 and potentially other plant TIR domains. Recent structures
232 of SNC1^{TIR} and RPP1^{TIR} [59,60] revealed both AE and DE self-association interfaces within the
233 crystal structures. Both interfaces also appear to control self-association, and we speculate that these
234 interfaces may facilitate SCAF in the plant TIR domains (Figure 2).

235

236 **4. SELF-ASSOCIATION AND HOMOTYPIC ASSOCIATION OF TIR DOMAINS IN BACTERIAL PROTEINS**

237 A common self-association interface has been observed in the available crystal structures of
238 bacterial TIR domains, PdTLP^{TIR} from the non-pathogenic *Paracoccus denitrificans* [61] and TcpB
239 from the pathogenic *Brucella melitensis* [24,53,62] (Figure 3, Table S1). The dimer interfaces in
240 both involve the DD and EE loops (different interface than the DE interface in plant TIR-domains)
241 and leave the BB loops exposed on the surface of the molecules. While TcpB^{TIR} associates
242 transiently, full-length TcpB forms a stable dimer [62] and in one of the crystal structures, a helix
243 corresponding to the sequence N-terminal to the TIR domain has been found to stabilize the
244 interaction [24]. PdTLP and TcpB, as well as a number of other bacterial TIR-domain proteins,
245 interact with MyD88, and some have been shown to interact with other mammalian TIR domains,
246 including MAL^{TIR} and TLR4^{TIR}, and interfere with NF-κB signaling [21,42,62].

247

248 **5. RECONCILIATION OF STRUCTURAL DATA IN THE CONTEXT OF HIGHER-ORDER ASSEMBLY**
249 **FORMATION**

250 While common trends in association modes are emerging in plant and bacterial TIR domains, this is
251 still not the case in animal TIR domains, despite the more extensive research. What could be the
252 possible reasons for this? For the domains functional in innate immunity signaling, the associations
253 need to be weak by design, so that responses are not too easily triggered in the absence of a
254 pathogen or danger inducer. The specific conditions required for crystallization may therefore easily
255 destabilize these interactions. Furthermore, the domains may have a tendency to assemble into
256 higher-order oligomers not compatible with crystal formation. Indeed, higher-order assembly is an
257 emerging feature of signaling in diverse innate immunity pathways. Protein domains from the DD
258 family, in particular, appear to be able to form large, often open-ended helical structures [63,64].
259 Signaling through cooperative assembly formation (SCAF) explains the ultrasensitive, all-or-none
260 response that is required in immune responses.

261 We propose that the available data on TIR-domain interactions can be reconciled by the
262 hypothesis that TIR domains that function in immunity pathways signal by cooperative assembly
263 formation (SCAF). The structures available to date likely provide snapshots into this assembly, but
264 the structures may, for reasons outlined above, vary in their biological relevance. Reconstitution of
265 stable complexes and their structural analysis, in combination with complementary cell biology
266 approaches, should reveal the interactions relevant to the signalosomes that occur *in vivo*.
267

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277

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- 346 Independent publications by three groups reported the structures of *B. melitensis* TcpBTIR
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482 An attempt to compare different higher-order assemblies, including various signalosomes,
483 amyloids and cellular granules, at structural, dynamic and regulatory levels.

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485 **FIGURE LEGENDS**

486

487 **Box 1. (a)** In the classical concept of receptor-mediated signaling, the activated receptor (R; for
488 example, activated by binding to the ligand L, blue) initiates signal transduction inside the cell
489 through successive steps of activation of signaling proteins (E; for example, enzymes that perform
490 post-translational modifications, such as protein kinases, or enzymes that produce second
491 messengers, such as adenylyl cyclases). This leads to signal amplification in a cascade-like fashion.
492 Red and green represent inactive and activated proteins, respectively. **(b)** In the case of signaling by
493 cooperative assembly formation (SCAF), the activated receptor initiates signal transduction through
494 higher-order assembly formation, which involves cooperative interactions with adaptor proteins (A)
495 and eventually enzymes (E) to form a signalosome. The large assembly can lead to rapid activation
496 of enzymes such as protein kinases or proteases through proximity-induced activation. The
497 cooperativity is the result of conformational changes and new binding sites generated by the
498 assembly architecture. SCAF appears to operate in most innate immunity pathways, including the
499 ones involving TIR domains. Most higher-order assemblies characterized to date are mediated by
500 members of the death-domain (DD) fold (DD, CARD, PYD, death-effector domain). The DD-
501 mediated helical assembly containing 6 MyD88 (shades of red), 4 IRAK4 (shades of green) and 4
502 IRAK2 (shades of blue) DDs [50] is shown as an example in **(c)** in cartoon representation.

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504

505 **Figure 1.** Representative TIR:TIR domain interactions based on structures of mammalian TIR
506 domains.

507 **(a)** Crystal contact-based TIR-domain dimers [26,30,37,40,43] (PDB ID 4W8H). The protomers
508 depicted on the right are all shown in analogous orientations.

509 **(b)** Superposition of one of the protomers from all the dimers shown in (a). The superimposed
510 protomer of the TLR2^{TIR} is shown in surface representation), with the other protomer from all the

511 TIR domains shown in different colors in ribbon representation.

512 (c) Head-to-tail arrangement of TIR domains in the crystals of MyD88^{TIR} [42] and TRR2^{TIR} (PDB
513 ID 4W8G and 4W8H).

514 (d) Two stranded parallel head-to-tail arrangement of TIR domains in the crystals of TRR2^{TIR} (PDB
515 ID 4W8G).

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517

518 **Figure 2.** TIR:TIR domain interactions mediated by the DE and AE interfaces in plant TIR-domain
519 proteins.

520 (a) Crystal contact-based TIR-domain dimers observed for the L6^{TIR} (blue) [17] and the
521 heterodimer of RPS4^{TIR} (dark green) and RRS1^{TIR} (green) [18], revealing the DE and AE interfaces,
522 respectively.

523 (b) Superimposed RPS4^{TIR} and L6^{TIR} dimers, revealing that the DE and AE interface can coexist.

524 (c) A hypothetical AE and DE interface-mediated assembly of plant TIR domains (individual
525 domains are shown in different colours). Note that this model does not account for other domains in
526 NLR proteins, such as the NB and LRR domains, which could influence the arrangement and
527 stoichiometry of predicted assemblies of plant NLRs, based on comparisons with the related
528 mammalian NLRs [14].

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530

531 **Figure 3.** TIR:TIR domain interactions in bacterial TIR-domain proteins. The crystal structures of
532 PdTLP^{TIR} (red) [61] and TcpB^{TIR} (blue) [24] reveal an analogous dimer interface. In one of the
533 structures of TcpB^{TIR} (PDB ID 4LZP) [24], the dimer is stabilized by a helix corresponding to the
534 sequence N-terminal to the TIR domain (light blue).

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536

537 **Table S1**

538

539 **Reports on the characterization of homotypic TIR-domain interactions.** Only binary
 540 interactions are listed in the table in cases where higher-order complexes have been analyzed in the
 541 original publications.
 542

Interacting TIR domains	Organism	Interface and interaction mode	Methods	Reference
Animal TIR domains				
TLR2 ^{TIR} -C713S:TLR2 ^{TIR} -C713S	<i>Homo sapiens</i>	Asymmetric dimer; involves α B, α C, α D, CD and DD (molecule A) and α B and BB (molecule B)	X-ray crystallography, mutagenesis	[33]
IL-1RAPL ^{TIR} :IL-1RAPL ^{TIR}	<i>Homo sapiens</i>	Symmetric dimer; involves α B, α C and α D	X-ray crystallography, mutagenesis	[30]
TLR2 ^{TIR} : MyD88 ^{TIR}	<i>Homo sapiens</i>	Involves BB and α A of both molecules	Computational docking, mutagenesis	[39]
TLR2 ^{TIR} :TLR2 ^{TIR} , MyD88 ^{TIR} :MyD88 ^{TIR}		Symmetric dimer; involves α E		
TLR1 ^{TIR} :TLR2 ^{TIR}	<i>Homo sapiens</i>	Two interacting regions; region I: involves TLR1 BB, TLR2 DD; region II: involves TLR1 α A (His646) and α C, TLR2 CD (Asn700)	Mutagenesis, computational docking	[44]
TLR4 ^{TIR} :TLR4 ^{TIR}	<i>Homo sapiens</i>	Symmetric dimer; involves BB	Modeling, docking, mutagenesis	[31]
TLR10 ^{TIR} :TLR10 ^{TIR}	<i>Homo sapiens</i>	Symmetric dimer; involves BB, DD, α B and α C	X-ray crystallography, mutagenesis	[40]
MyD88 ^{TIR} :MAL ^{TIR}	<i>Homo sapiens</i>	Two interacting sites on MyD88 (site 1 corresponds to BB (R196) and site 2 to α E)	NMR spectroscopy, mutagenesis, docking	[32]

		(R288)		
TLR4 ^{TIR} :TLR4 ^{TIR}	<i>Homo sapiens</i>	Symmetric dimer: involves BB, DD, α C	Computational docking and modeling	[45]
TLR7 ^{TIR} :TLR7 ^{TIR}		Asymmetric dimer: involves BB (molecule A), α E (molecule B)		
MyD88 ^{TIR} :MyD88 ^{TIR}		Symmetric dimer: involves BB, α C		
TLR4 ^{TIR} :SIGIRR ^{TIR}		3 patches; patch 1: involves TLR4 CD, and BB, SIGIRR α B; patch 2: involves TLR4 α B and α C, SIGIRR α C; patch 3: involves TLR4 BB, SIGIRR α D		
TLR7 ^{TIR} :SIGIRR ^{TIR}		Involves SIGIRR BB and α B, TLR7 α E, CD, β D, β E and DE		
MyD88 ^{TIR} :SIGIRR ^{TIR}		Involves MyD88 BB, and α C, SIGIRR BB, AA and α C		
MAL ^{TIR} :MAL ^{TIR}	<i>Homo sapiens</i>	Symmetric dimer: involves α C, α D	X-ray crystallography, docking, mutagenesis	[37]
MAL ^{TIR} :MyD88 ^{TIR}		Involves MAL D96 (AA) and S180 (DD), MyD88 R196 (BB)		
TLR4 ^{TIR} :TLR4 ^{TIR}	<i>Homo sapiens</i>	Symmetric dimer: involves BB, α C	Molecular dynamics (MD) simulations, molecular docking	[46]
TLR2 ^{TIR} :TLR1 ^{TIR}		Asymmetric dimer: involves TLR2 DD,		

		TLR1 BB		
TLR2 ^{TIR} :TLR6 ^{TIR}		Involves TLR2 DD, TLR6 BB		
ST2L ^{TIR} :MAL ^{TIR}		Involves ST2L AB and BB, MAL BB, β A and β B		
ST2L ^{TIR} :MyD88 ^{TIR}		Involves ST2L BB, AA and α A, MyD88 BB, α A		
MAL ^{TIR} :MAL ^{TIR}	<i>Homo sapiens</i>	Symmetric dimer: involves α C, α D	X-ray crystallography, mutagenesis	[41]
TLR4 ^{TIR} :TLR4 ^{TIR}	<i>Homo sapiens</i>	Symmetric dimer: involves BB, DD, α C	Mammalian protein-protein interaction trap (MAPPIT), homology modeling, mutagenesis	[28]
TLR4 ^{TIR} :MAL ^{TIR} , TLR4 ^{TIR} :TRAM ^{TIR}		Involves TLR4 α A, α B, BB, BC		
MAL ^{TIR} :MAL ^{TIR}	<i>Homo sapiens</i>	Asymmetric dimer: involves DD, DE, α D (molecule A), N-terminal region (molecule B)	X-ray crystallography	[47]
MAL ^{TIR} :MAL ^{TIR}	<i>Homo sapiens</i>	Symmetric dimer: involves α C, α D	Random mutagenesis, MAPPIT	[27]
MAL ^{TIR} :MyD88 ^{TIR}		Involves MAL AB loop and two surface areas (area 1: Q135, W156; area 2: Y195, R215)		
MAL ^{TIR} :TLR4 ^{TIR}		Involves MAL AB loop and three surface areas (area 1: Q135, W156; area 2: Y195, R215; area 3: Q153, R184, R192)		
TRAM ^{TIR} :TRAM ^{TIR}	<i>Homo sapiens</i>	Symmetric dimer: involves BB, α C	NMR spectroscopy, mutagenesis, docking	[36]

MyD88 ^{TIR} :MyD88 ^{TIR}	<i>Homo sapiens</i>	Symmetric dimer: involves β A, AA, α A, AB, CD, BB, α C	Site-directed mutagenesis, computational modeling	[48]
MyD88 ^{TIR} :MyD88 ^{TIR}	<i>Homo sapiens</i>	Two asymmetric dimers: dimer 1 involves α C, α D (molecule A), α A, EE and α E (molecule B); dimer 2 involves α B, BB (molecule A), DD, α D, EE and α E (molecule B)	X-ray crystallography	[42]
MAL ^{TIR} :MAL ^{TIR}	<i>Homo sapiens</i>	Symmetric dimer: involves α C, α D	X-ray crystallography, mutagenesis	[53]
TLR6 ^{TIR} :TLR6 ^{TIR}	<i>Homo sapiens</i>	Symmetric dimer: involves CD, DD, α B α C	X-ray crystallography, MALS	[43]
TLR4 ^{TIR} :TLR4 ^{TIR}	<i>Homo sapiens</i>	Two symmetric dimers; both involve BB	Modeling, <i>in silico</i> mutagenesis	[29]
MAL ^{TIR} :MAL ^{TIR}		Symmetric dimer: involves AB		
TRAM ^{TIR} :TRAM ^{TIR}		Symmetric dimer: involves BB		
ST2 ^{TIR} :TLR4 ^{TIR} , ST2 ^{TIR} :TRIF ^{TIR}	<i>Homo sapiens</i>	Involves BB	Modeling, <i>in silico</i> mutagenesis	[38]
MyD88 ^{TIR} :MyD88 ^{TIR}	<i>Homo sapiens</i>	Asymmetric dimer: involves BB (molecule A), α E (molecule B); symmetric dimer: involves α D, α C	MAPPIT, mutagenesis, docking	[35]
TLR4 ^{TIR} :TLR4 ^{TIR}	<i>Mus musculus</i>	Asymmetric dimer: involves BB (molecule A), α E (molecule B)	Decoy peptides, modeling	[34]

TRR-2 ^{TIR} :TRR-2 ^{TIR}	<i>Hydra magnipapillata</i>	Asymmetric dimer: involves BB (molecule A), βD, βE, DE, αE (molecule B); symmetric dimer: involves αB, αC, αD	X-ray crystallography	Weisse & Scheidig, unpublished; PDB ID 4W8G
TRR-2 ^{TIR} :TRR-2 ^{TIR}	<i>Hydra magnipapillata</i>	Asymmetric dimer 1: involves BB (molecule A), βD, βE, DE, αE (molecule B); asymmetric dimer 2: involves αA, αE (molecule A), αB, αC, αD (molecule B)	X-ray crystallography	Weisse & Scheidig, unpublished; PDB ID 4W8H
Plant TIR domains				
L6 ^{TIR} :L6 ^{TIR}	<i>Linum usitatissimum (flax)</i>	Symmetric dimer: involves αD ₁ , αD ₃ , αE, βE, DE, EE (DE interface)	X-ray crystallography, MALS, analytical ultracentrifugation (AUC), yeast two-hybrid (Y2H) analysis	[17]
RRS1 ^{TIR} :RPS4 ^{TIR}	<i>Arabidopsis thaliana</i>	Pseudo-symmetric dimer: involves αA, αE, EE (of both RRS1 ^{TIR} and RPS4 ^{TIR}) and DD (RRS1 ^{TIR}) (AE interface)	X-ray crystallography, MALS, SAXS, Y2H analysis	[18]
RPS4 ^{TIR} :RPS4 ^{TIR}	<i>Arabidopsis thaliana</i>	Symmetric dimer: involves αA, αE, EE (AE interface)	X-ray crystallography, MALS, SAXS, AUC, Y2H analysis	[18]
RRS1 ^{TIR} :RRS1 ^{TIR}	<i>Arabidopsis thaliana</i>	Symmetric dimer: involves αA, αE, EE (AE interface)	X-ray crystallography	[18]
RPV1 ^{TIR} :RPV1 ^{TIR}	<i>Muscadinia rotundifolia (wild grapevine)</i>	Symmetric dimer: involves αA, αE, EE (AE interface)	X-ray crystallography	[58]

SNC1 ^{TIR} :SNC1 ^{TIR}	<i>Arabidopsis thaliana</i>	Two dimer interfaces; interface 1 (AE interface), involves αA , αE , EE; interface 2 (DE interface): involves αD_1 , αE , βE , DE, EE	X-ray crystallography	[59]
SNC1 ^{TIR} :SNC1 ^{TIR}	<i>Arabidopsis thaliana</i>	Two dimer interfaces; interface 1 (AE interface), involves αA , αE , EE; interface 2 (DE interface): involves αD_1 , αE , βE , DE, EE	X-ray crystallography, MALS, SAXS	[60]
RPP1 ^{TIR} :RPP1 ^{TIR}	<i>Arabidopsis thaliana</i>	Two dimer interfaces; interface 1 (AE interface), involves αA , αE , EE; interface 2 (DE interface): involves αD^1 , αE , βE , DE, EE	X-ray crystallography, MALS	[60]
Bacterial TIR domains				
PdTLP ^{TIR} :PdTLP ^{TIR}	<i>Paracoccus denitrificans</i>	Symmetric dimer: involves DD, EE	X-ray crystallography, hydrogen/deuterium exchange mass spectrometry (DXMS)	[61]
TcpB ^{TIR} :TcpB ^{TIR}	<i>Brucella melitensis</i>	Involves DD, EE, αC , αD	X-ray crystallography, MALS	[24]
TcpB ^{TIR} :TcpB ^{TIR}	<i>Brucella melitensis</i>	Symmetric dimer: involves DD, EE	X-ray crystallography, SAXS, MALS	[62]

TcpB ^{TIR} :TcpB ^{TIR}	<i>Brucella melitensis</i>	Symmetric dimer: involves DD, EE	X-ray crystallography, DXMS	[53]
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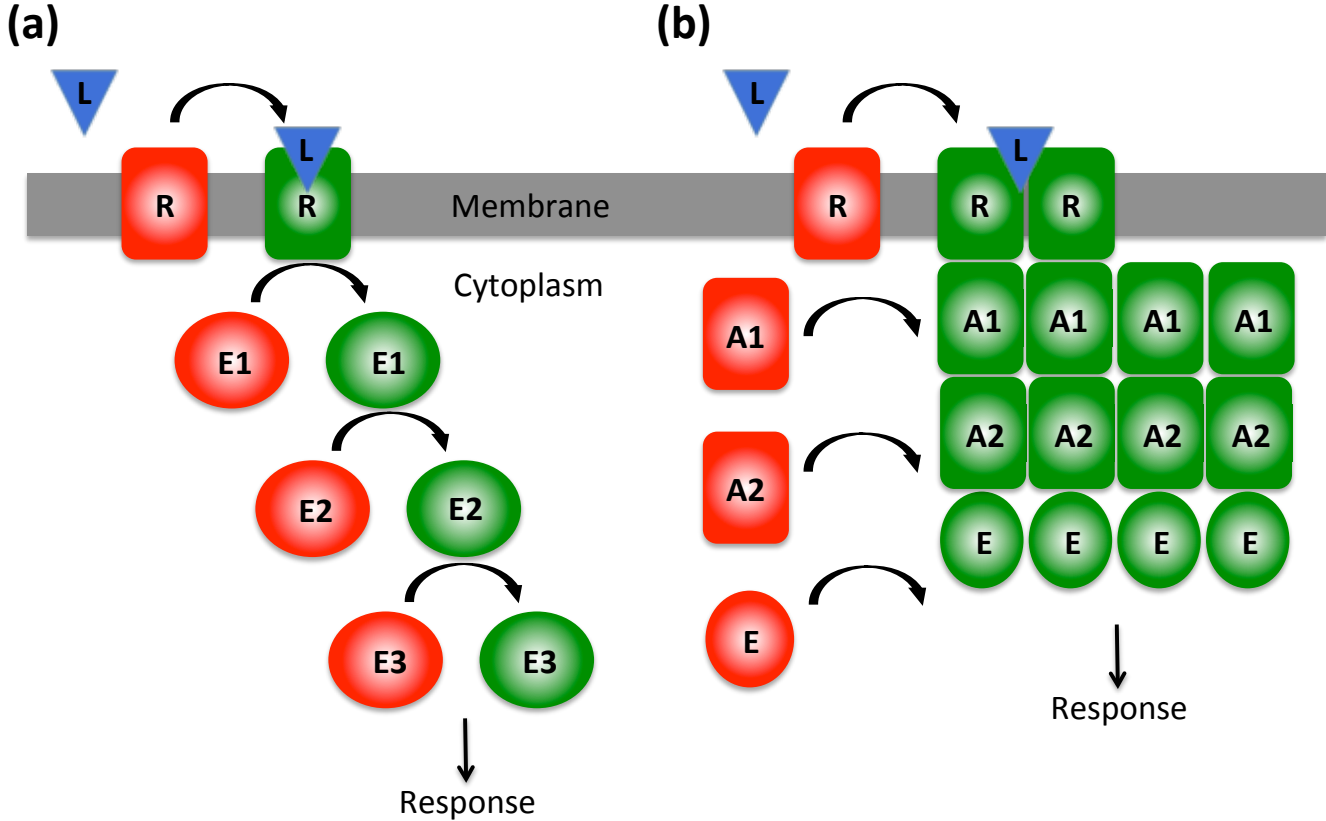
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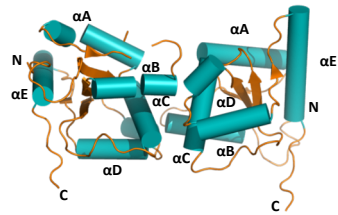
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Box 1

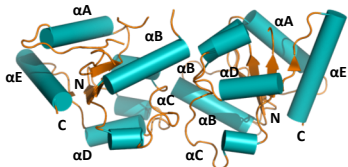


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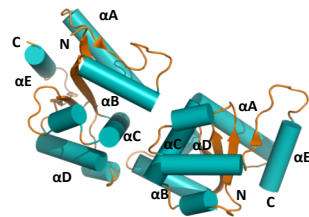
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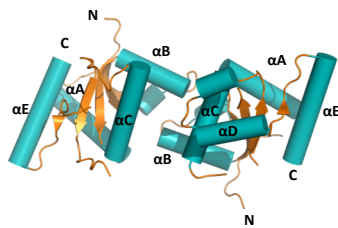
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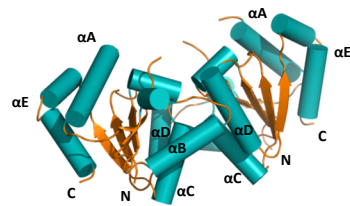
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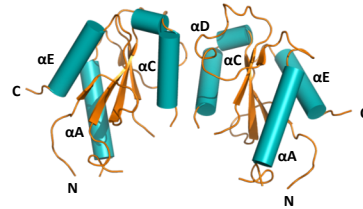
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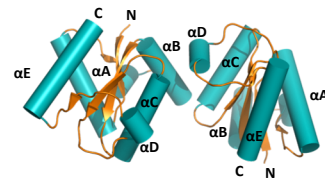
IL-1RAPL



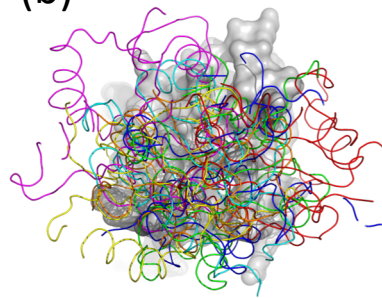
MAL



TRR2

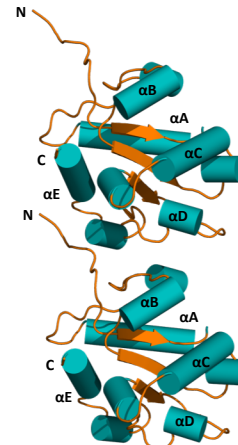


(b)

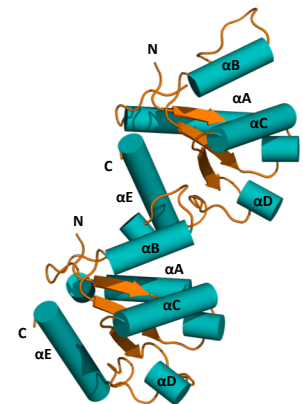


(c)

MyD88



TRR2



(d)

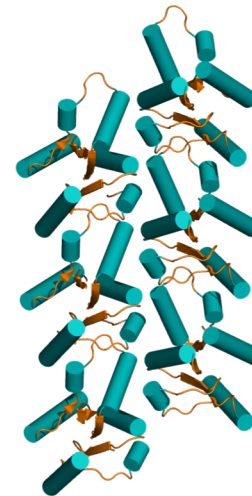
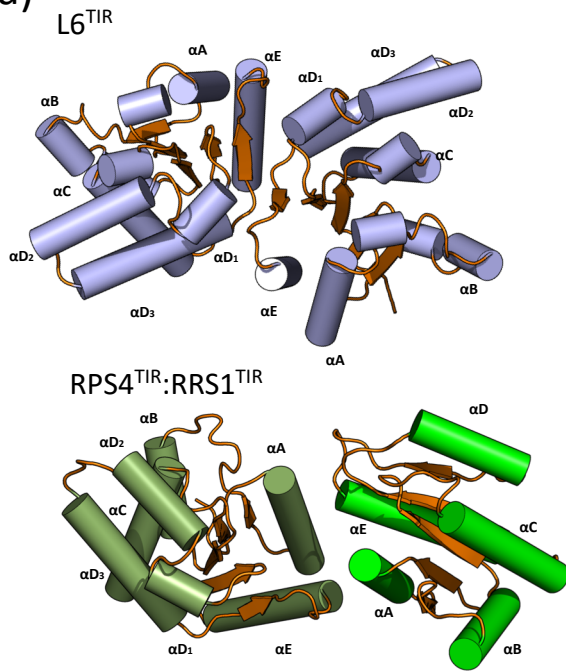


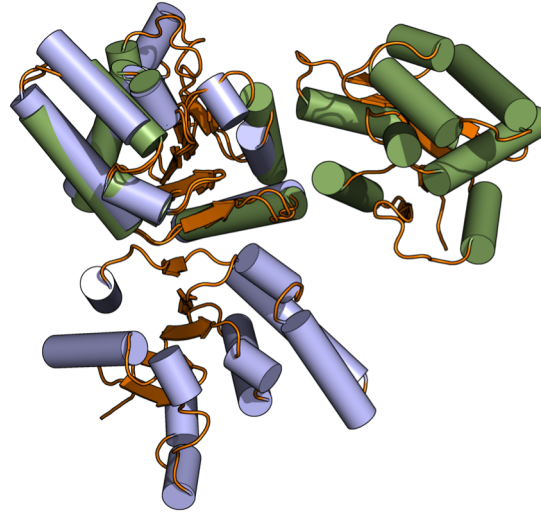
Fig. 2

(a)



(b)

DE ($L6^{TIR}$) and AE ($RPS4^{TIR}$) interfaces



(c)

A hypothetical AE/DE interface-mediated oligomer

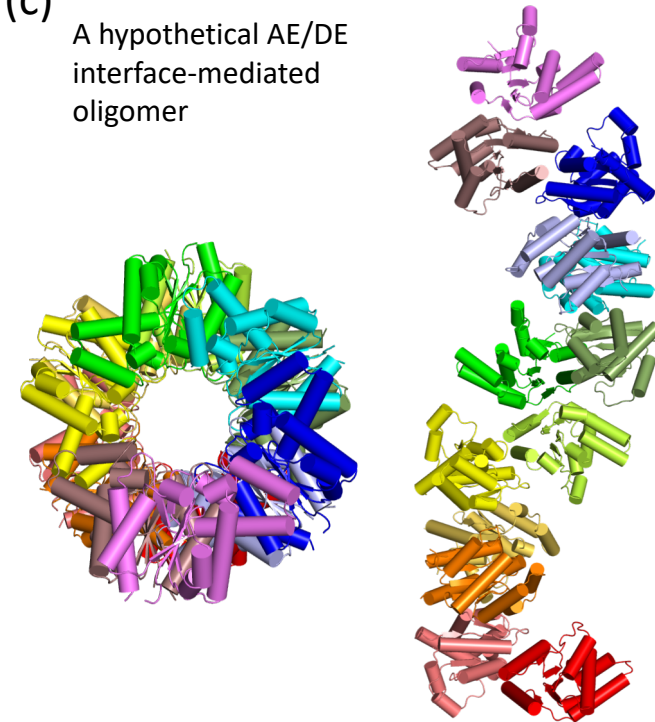
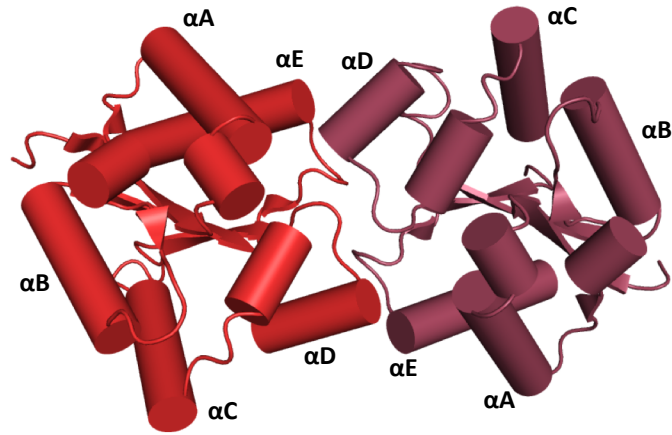


Fig. 3

PdTLPTIR



TcpB^{TIR}

